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RESPIRATORY REFINEMENTS IN THE MYGALOMORPH SPIDER *GRAMMOSTOLA ROSEA* WALCKENAER 1837 (ARANEAE, THERAPHOSIDAE)

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ABSTRACT. In this study we hypothesized that *Grammostola rosea* Walckenaer 1837, an active predator of large size that depends on its two paired book lungs for respiration, would have a refined low energy strategy based on its thin air-hemolymph barrier. The morphology of book lungs and the oxygen consumption at 20° and 30° C under normal and starvation conditions were studied. The oxygen consumption was low compared to that expected for spiders from the allometric relationship, 0.027 ± 0.01 ml O_2 g^{-1} h^{-1} (average ± standard deviation), and it was depressed at 30° C under starvation. The harmonic mean thickness of the air-hemolymph barrier was 0.14 ± 0.03 µm, the respiratory surface density was 122.99 ± 35.84 mm^{-1}, and the book lung volume ranged from 12.2 to 37.5 mm^3. With these parameters a high oxygen diffusion capacity was estimated. The combination of low resting oxygen consumption and high pulmonary oxygen conductance results in very low gradients of partial oxygen pressures across the air-hemolymph barrier (0.12–0.16 kPa) required to satisfy the resting oxygen demands.

Keywords: Oxygen consumption, book lungs, mygalomorph spider

In mygalomorph spiders, respiration involves the movement of gases across an exchange surface and their combination with the circulating respiratory pigment hemocyanin (Anderson & Prestwich 1982). The book lungs are two paired organs located within the abdomen of spiders in an inextensible chitinous cavity called the atrium (Foelix 1996). The respiratory organ is composed of a series of flattened air-filled cuticular plates, the lamellae, which are projected into a hemolymphatic sinus. Gas exchange occurs across a thin cuticle-hypodermis barrier separating the gases of the atrium from the hemolymph. The book lungs constrain oxygen consumption in spiders, which exhibit resting metabolic rates about half those measured for other poikilothermic animals of equal mass (Anderson 1970; Greenstone & Bennett 1980). This low oxygen consumption has been considered an unusual energetic adaptation of sit and wait predators. Their metabolic performance is improved further by an ability to depress metabolic rates below usual resting levels during transient periods of starvation (Ito 1964; Miyashita 1969; Nakamura 1972; Anderson 1974; Humphreys 1977).

Several studies have attempted to relate foraging styles to oxygen consumption (Carrel & Heathcote 1976; Angersbach 1978; Greenstone & Bennett 1980; Paul et al. 1987; Strazny & Perry 1984; Schmitz & Perry 2001). The active jumping spider *Salticus scenicus* (Salticidae) only requires an air-hemolymph PO_2 gradient between 0.22 to 0.26 kPa for a sustained metabolic rate value of 0.312 ml O_2 g^{-1} h^{-1} at rest.

In this study we hypothesized that *Grammostola rosea* Walckenaer 1837, an active predator of large size which depends entirely on its two paired book lungs, has a low energy strategy based on large respiratory surface area and a thin air-hemolymph barrier.

METHODS

Six adult individuals of *G. rosea* (Body mass = $M_b = 18.5 \pm 6.2$ g) were kept in individual containers for 7 days at natural lab temperatures to ensure acclimation conditions prior to measurements. Water was periodically added to a cotton swab placed at the end of the cage. Several larvae of *Tenebrio molitor* were added daily as an *ad lib.* source of food. The photoperiod was kept at 12 h:12 h L:D. After 2 wk metabolic rate (MR) was measured at 20° and 30° C. The same measurements were repeated after 3 wk of starvation.

All metabolic trials were performed during the day, which corresponds to the resting phase in this species. Rates of oxygen consumption ($\dot{V}O_2$) were used as a measure of MR, and were determined using "closed system" metabolic chambers (Vleck 1987). Animals were weighed to the nearest mg on an analytical balance and then placed individually inside a chamber of 60 cm^3. Small granules of CO_2-absorbent Baralyme™ and water absorbent Drierite™ were added to each chamber in a compartment isolated from the spider. The chambers were sealed from the atmosphere and placed for 2 h in a temperature and light controlled incubator during the resting phase. Three blank chambers served as controls for each series of measurements. After two hour long experiments we injected the air from each chamber into a Tygon™ tube connected to the O_2 analyzer. At the end of the measurement interval O_2 concentrations were determined using an Oxygen Analysis System FC 10a (Sable System International, Henderson, NV, USA), supplied with barometric pressure compensation. Output from the analyzer was recorded by a computer using EXPEDATA program (Sable's data acquisition system). Rates of oxygen consumption were calculated using:

$$\dot{V}_{O2} = \frac{V \cdot (FI_{O2} - FE_{O2})}{(1 - FE_{O2}) \cdot t},$$

where V is the initial volume of dry, CO_2-free air in the chamber at STP, FI_{O2} and FE_{O2} are the O_2 fractions within the chamber at the start and the end of incubation, respectively, and t is the duration of incubation. Comparisons among oxygen consumption at different temperatures and at the two experimental feeding conditions were performed using non parametric two-way ANOVA (Friedman test).

Three of the spiders (M_b = 13.4 ± 2.65 g) were sacrificed, and their book lungs were carefully extracted and immersed in 2.3% glutaraldehyde in phosphate buffer at 4° C for a minimum of 2 h. Next, tissues were processed for routine electronic transmission microscopy. Briefly, two randomly chosen pieces were obtained from each book lung. The pieces were washed with buffer and post-fixed with 1% osmium tetroxide for 1 h at 4° C. Tissues were then dehydrated in graded concentrations of alcohol and infiltrated and embedded in epoxy resin constructing cubes of 2–3 mm^3 that were sectioned in semi-thin sections of 1 μm. Tissue samples were stained with 1% toluidine blue. Ultra thin sections of 60–90 nm of thickness were made and mounted on copper mesh grids. These sections were contrasted with Pb-citrate. The semi-thin sections were contrasted with hematoxiline-eosine. Sections were studied using optical and transmission electron microscopy (JEOL/JEM 100SX). Six sections were photographed, digitized and six semi-thin and six ultra-thin sections per individual were analyzed using Scion Image Software. The total harmonic mean thickness of the air-hemolymph barrier (τ_h (μm)) and those of the cuticle and hypodermis layers were estimated by a stereological method in a square lattice grid as suggested by Weibel (1970/71) and Maina (2002):

$$\frac{1}{\tau_h} = \frac{3}{2} \cdot \frac{\sum_{j=1}^{m} f_j \cdot \frac{1}{l_j}}{\sum_{j=1}^{m} f_j},$$

where l_j is the mid-value of intercept length of linear probes, f_j the frequency of class j and m the number of classes.

The respiratory surface density (RS_d (mm^{-1})), the respiratory surface area (mm^2) per lung volume unit (mm^3), was estimated by means of line-intersection stereological method (Weibel 1970/71):

$$RS_d = \frac{2N}{1/2 \cdot P_T \cdot Z},$$

where N is the number of intersections between line probes of length Z with the respiratory surface and P_T is the number of testing points.

Two spiders (M_b: A1 = 13.62 g; A2 = 16.8 g) were selected to estimate the volume of the book lung (i.e., respiratory zone of the atrium). These spiders were killed and then the entire opisthosoma was extracted, fixed and contrasted. Equidistant semi thin sections of 6 μm were taken along the entire lung zone. Each section was observed and photographed under a light microscope. Each image was analyzed, determining the sectional area of the respiratory zone (Ai), and then, the total volume (BLV) was estimated based on the Cavalieri principle (Howard & Reed 2005), using:

$$BLV = \sum_i A_i \cdot d_i,$$

where d_i is the distance between the sections, and in our case a constant value of d_i = 6 μm.

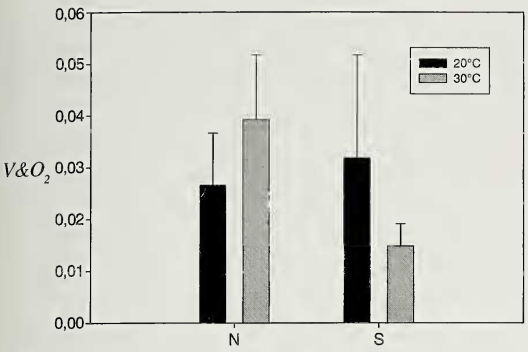

Figure 1.—Resting metabolic changes of *Grammostola rosea* at 20° (black bars) and 30° C (white bars), under normal (N) and starvation (S) conditions. $\dot{V}O_2$ = oxygen consumption (ml O_2 h^{-1} g^{-1}). Means + 1 SD are shown.

From those values the oxygen diffusion capacity (D_tO_2) that represents the oxygen conductance of each layer of the air-hemolymph barrier was estimated by:

$$DO_i = \frac{\kappa \cdot RS_d \cdot BLV}{\tau_h},$$

where DO_i is the oxygen diffusion capacity of a layer and κ is the Krogh's diffusion coefficient: 1.28×10^{-8} cm^2 min^{-1} kPa^{-1} ($= 76.8 \times 10^{-8}$ cm^2 h^{-1} kPa^{-1}) for the cuticle and 2.05×10^{-7} cm^2 min^{-1} kPa^{-1} ($= 123.0 \times 10^{-8}$ cm^2 h^{-1} kPa^{-1}) for hypodermis (Schmitz & Perry 2001). Because the oxygen conductance is the inverse value of the resistance (D_tO_2 = 1/R), and cuticle and hypodermis are disposed in a series array, the total D_tO_2 of both layers was computed by:

$$\frac{1}{D_tO_2} = \frac{1}{DO_c} + \frac{1}{DO_h},$$

where DO_c and DO_h are the oxygen diffusion capacities of the cuticle and hypodermis, respectively. Finally, the required gradient of oxygen partial pressures between the gases of the atrium and the hemolymph for a particular value of oxygen consumption ($\dot{V}O_2$*) was estimated by $\Delta PO_2 = \dot{V}O_2*/D_tO_2$.

RESULTS

Grammostola rosea showed a low $\dot{V}O_2$ at 20° C, 0.027 ± 0.01 ml O_2 g^{-1} h^{-1}, with a Q_{10} = 1.65 ± 0.78 between 20° and 30° C of environmental temperature. The metabolic rate was affected by the different conditions (χ^2_3 = 9.72, P = 0.02) and this was due to a decrease in $\dot{V}O_2$ at 30° C in the starvation condition (P < 0.05 in planned comparisons) and marginally due to the temperature factor (P = 0.07; Figure 1).

Figure 2.—Semi thin section of the book lung of *Grammostola rosea* (10×). Scale bar = 1 mm.

Table 1.—Metabolic and structural respiratory parameters in two spiders, *Grammostola rosea*. M_b = body mass, $\dot{V}O_2$ = oxygen consumption at 20° C, BLV = book lung volume, D_tO_2 = oxygen diffusion capacity, $DtO2^m$ = mass-specific oxygen diffusion capacity, and ΔPO_2 = required gradient of partial oxygen pressures between air and hemolymph to support these $\dot{V}O_2$ values.

Spider	M_b (g)	$\dot{V}O_2$ (ml O_2 h^{-1} g^{-1})	BLV (mm^3)	D_tO_2 (cm^3 h^{-1} kPa^{-1})	$D_tO_2{}^m$ (cm^3 h^{-1} kPa^{-1})	ΔPO_2 (kPa)
A1	13.62	0.0372	12.2	3.165	0.233	0.160
A2	16.8	0.0659	37.5	9.277	0.552	0.119

The respiratory surface density was RS_d = 122.99 ± 35.84 mm^{-1} and the harmonic mean thickness of the air-hemolymph barrier was τh = 0.14 ± 0.03 μm (Fig. 2). The cuticle represents 22.2 ± 10.3% of the total thickness of the barrier. The book lung volume of the spiders A1 and A2 were 12.2 mm^3 and 37.5 mm^3, respectively. Their respiratory surface area ($RS_d \times BLV$) was estimated to vary between 1500.5 mm^2 to 4612.1 mm^2. These spiders showed a $\dot{V}O_2$ = 0.037 ml O_2 h^{-1} g^{-1} and $\dot{V}O_2$ = 0.066 ml O_2 h^{-1} g^{-1} at 20° C. Considering these values, oxygen diffusion capacities for the cuticle 3.85 cm^3 h^{-1} kPa^{-1} and 11.28 cm^3 h^{-1} kPa^{-1}, and oxygen diffusion capacities for the hypodermis 17.86 cm^3 h^{-1} kPa^{-1} and 52.24 cm^3 h^{-1} kPa^{-1} were obtained for A1 and A2 spiders respectively. The oxygen diffusion capacities of the total barrier (D_tO_2) are given in Table 1.

DISCUSSION

Grammostola rosea showed refined morphological characteristics in its book lungs. Their thin air-hemolymph barrier, combined with appropriate values of respiratory surface density and book lung volume, results in high oxygen diffusion capacities allowing a good oxygen delivery even at low oxygen pressures.

The respiratory surface area of *G. rosea* was lower than that reported for *Aphonopelma* (*Eurypelma*) *californicum* {(Ausserer 1871) Theraphosidea, nomina dubium (Platnick 2006)} (6400 mm^2; Focke 1981). Compared with other spiders, the RS_d was lower than that for the jumping spider *Salticus scenicus* (Clerck 1757) (Salticidae) and lower than *Tegenaria* spp. (Agelenidae) 210–250 mm^{-1} and 355–390 mm^{-1}, respectively. However, the thickness of the air-hemolymph barrier in *G. rosea* (0.14 μm) was thinner than that of these spiders, 0.17–0.18 μm in *S. scenicus* and 0.4 μm reported in *Tegenaria* spp. (Strazny & Perry 1984; Schmitz & Perry 2001). The resulting D_tO_2 (0.233 cm^3 h^{-1} kPa^{-1}) was similar to that of *Tegenaria* spp. 0.258–0.552 cm^3 h^{-1} kPa^{-1} but lower than that of *S. scenicus* 0.720–0.984 cm^3 h^{-1} kPa^{-1}. The required gradient of partial oxygen pressures between air and hemolymph to support the resting $\dot{V}O_2$ at 20° C (ΔPO_2) was 0.119 to 0.160 kPa, which is close to the required 0.22–0.26 kPa required by *S. scenicus* at rest (Schmitz & Perry 2001). The lower ΔPO_2 requirement of *G. rosea* compared with that of *S. scenicus* in spite of their lower D_tO_2 arises from its lower mass specific oxygen consumption and represents a value of about 2% of that reported in mammals (7.5 kPa). The required $\Delta PO2$ in *G. rosea* is also lower than 0.7 kPa, a value estimated across the lung barrier in *Aphonopelma californicum* during rest (Angersbach 1978; Paul et al. 1987). Considering that spiders have aerobic scopes between 5 and 8 (Seymour & Vinegar 1973; Herreid 1981; Anderson and Prestwich 1985), the ΔPO_2 required by an active individual of *G. rosea* could reach 0.8 kPa and a maximum of 1.28 kPa. A required ΔPO_2 = 7 kPa was estimated across the walls of the lungs of *A. californicum* after activity (Angersbach 1978; Paul et al. 1987), a value near those usually found in mammals; however this estimation was performed assuming a thick air-hemolymph barrier (0.89 μm). If we replace that value by 0.2 μm, similar to *G. rosea* and other spiders, the required ΔPO_2 decreases to 1.59 kPa, similar to our result. Our results are lower but comparable to the range of 2.2 to 3.0 kPa estimated for the active jumping spider *S. scenicus* and the 2.4 kPa measured in the less active *Tegenaria* spp. during molting.

Grammostola rosea showed 63.3% of the expected oxygen consumption for spiders from the allometric relationship: log $\dot{V}O_2$ (μL h^{-1}) = −0.133 + log M_b (mg) (Greenstone & Bennet

1980), less than half of the values measured for other poikilothermic animals (Anderson 1970). The value of $\dot{V}O_2$ is similar to those of *Aphonopelma eutylenum* Chamberlin 1940 (Theraphosidae) (0.018 ml O_2 h^{-1} g^{-1}; Greenstone & Bennet 1980) and *A. californicum* (0.013 ml O_2 h^{-1} g^{-1}; Paul et al. 1987). Moreover, *G. rosea* showed depressed metabolic rates after a starvation period of three weeks, agreeing with results from other spiders (Ito 1964; Miyashita 1969; Nakamura 1972; Anderson 1974; Humphreys 1977). This metabolic depression was only evident at 30°C, probably due to the high energetic requirement derived from the exponential relationship between temperature and metabolism in ectothermic animals.

In Chile there are no studies on the population dynamics of this species but it is possible to find adults throughout the year. The reported metabolic and morphologic findings could account for a general lack of numerical responses to insect prey availability in this temperate zone (Greenstone 1978) and could be part of physiological adaptations to tolerate low or unpredictable food availability (McNab 1974), buffering spiders against the environmental fluctuations (Mediterranean weather) characteristic of their habitat in central Chile.

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